

A)

Query	Subject	Score	Identities (Query length)	Percentage	Expect
Query1	HORVU3Hr1G019850 chr3H:58407698-58410314 HC_G cytokinin	37.4	20/20 (20)	100	0.004
Query1	HORVU0Hr1G013800 chrUn:77795619-77830417 HC_G oligopeptide	31.9	17/17 (20)	100	0.17
Query1	HORVU5Hr1G095440 chr5H:598313214-598325798 HC_G tornado	31.9	17/17 (20)	100	0.17
Query1	HORVU5Hr1G095460 chr5H:598320669-598325900 HC_G oligopeptide	31.9	17/17 (20)	100	0.17
Query1	HORVU5Hr1G086250 chr5H:576150954-576183325 HC_G SAUR-like	28.3	15/15 (20)	100	2.1
Query1	HORVU3Hr1G046180 chr3H:306942032-306970001 HC_G Protein	28.3	15/15 (20)	100	2.1
Query1	HORVU4Hr1G059060 chr4H:493802120-493805262 HC_G ADP-ribosylation	28.3	15/15 (20)	100	2.1
Query1	HORVU5Hr1G086240 chr5H:576148495-576183422 HC_G SAUR-like	28.3	15/15 (20)	100	2.1
Query1	HORVU1Hr1G067230 chr1H:477443466-477449042 HC_G metacaspase	28.3	17/18 (20)	94	2.1
Query1	HORVU5Hr1G066370 chr5H:506740106-506766525 HC_G Copper-transporting	28.3	15/15 (20)	100	2.1

B)

	PAM	target sequence	
HORVU3Hr1G019850	<u>CCGCGGCGTCTCCTACGGCGCAC</u>	on-target	
HORVU0Hr1G013800	<u>CTGCGGCGTCTCCTACGGCGGCG</u>	no NGG PAM	
HORVU5Hr1G095440	<u>CTGCGGCGTCTCCTACGGCGGCG</u>	no NGG PAM	
HORVU5Hr1G095460	<u>CTGCGGCGTCTCCTACGGCGGCG</u>	no NGG PAM	
HORVU5Hr1G086250	<u>CGACCGCGTCTCCTACGGCGAGC</u>	no NGG PAM	
HORVU3Hr1G046180	<u>CGACCGCGTCTCCTACGGCGAGC</u>	no NGG PAM	
HORVU4Hr1G059060	<u>GGCAGCGTCTCCTACGGCGTCT</u>	no NGG PAM	
HORVU5Hr1G086240	<u>CGACCGCGTCTCCTACGGCGAGC</u>	no NGG PAM	
HORVU1Hr1G067230	<u>CAGCGGCGTGTCCTACGGCGCGC</u>	no NGG PAM	
HORVU5Hr1G066370	<u>CGACCGCGTCTCCTACGGCGAGC</u>	no NGG PAM	

Figure S4. Off-target analysis for *HvCKX1* guide sequence. (A) Table representing 10 best results of Blast search against barley genome sequence (http://webblast.ipk-gatersleben.de/barley_ibsc/viroblast.php; assembly_WGSMorex) and **(B)** sequence alignments of the respective regions covering guide RNA target sequence including PAM